

Clustering and signalling of cell receptors

Yu Shi *

Cavendish Laboratory, University of Cambridge, Cambridge CB3 0HE, United Kingdom

Abstract

As a response to ligand binding, transmembrane cell receptors often enhance their clustering, or oligomerization, during the signalling process. Here we present a statistical mechanical model which combines the aspects of clustering and signalling. In this model, receptors float on the surface, while for two neighboring receptors, there is an interaction energy dependent on their conformational states. On the other hand, ligand binding of a receptor shifts the energy difference between the two conformational states. Due to thermal fluctuation, the effects of clustering and signalling are statistical average quantities. This model reduces to a floating Ising model with a random field. We calculate the signalling in a grand canonical ensemble mean field approach, using Hubbard-Stratonovich transformation and replica method. Monte Carlo simulations are also performed. Essential biological features are obtained in our model.

Keywords: cell receptors, clustering, floating Ising model in a random field

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*Email address: ys219@cam.ac.uk

1. INTRODUCTION

On the surface of a cell, there are many transmembrane receptor molecules. They are “sensory machines” of the cell. Binding extracellular ligands changes the conformational states or, in our point of view, the probability distribution of the conformational states. Consequently a cascade of responses occurs inside the cell. This is called signalling, which is often quite sensitive. An example is bacterial chemotaxis [1]: through the detection by its receptors, a bacterium swims towards higher concentration of a chemical it likes or a lower concentration of a chemical it dislikes. In this case, thousands of receptors cluster together at a pole of the cell. Recently, based on detailed analyses on the experimental findings, I proposed that cooperative receptor-receptor interaction enhances the signalling, and constructed a statistical mechanical theory for this process [2–4]. My idea has since received considerable attention ¹. In [4], good consistency was found in comparing this theory with experimental results [5–10], and it was also suggested what kinds of experiment are needed.

In the case of many other cell receptors, however, usually they float on the cell membrane. A ubiquitous phenomenon is that ligand binding enhances the clustering of receptors during the signalling process. Investigations on this issue are widely reported for various receptors, for example, integrins, which attach cells to the extracellular matrix, tyrosine kinase receptors, cytokine receptors, growth factor receptors, tumor necrosis factor receptors, including death receptors, antigen receptors such as T-cell receptors, bone morphogenetic protein receptor, G-protein-coupled receptors, etc [11–19]. In our previous works [2–4], the position of each receptor is fixed, and there is an *a priori* cluster. In the present article, we shall consider statistical mechanics of floating receptors. Based on our model, it is proposed that signalling sensitivity and clustering enhancement, in certain cases, are likely two consequences of the same mechanism based on conformation-dependent local receptor-receptor interaction.

In Sec. 2, we give a detailed description of the model. A mean field calculation of the signalling, as a statistical average quantity, is given in Sec. 3. In Sec. 4, we discuss receptor clustering. Results of Monte Carlo simulations are given in Sec. 5. Finally a summary and discussion is made in Sec. 6.

2. THE MODEL

Consider a collection of receptors on a lattice. Fig. 1 is an illustrative configuration. Let the coordinate number be ν , which is 6 for a honeycomb lattice and is 4 for a square lattice. Suppose that the receptors can move around on the lattice. Hence at an instant, a receptor has $\leq \nu$ nearest-neighboring receptors. The conformational state of the receptor at lattice point i is represented as V_i , the value of which is either V^0 or V^1 , if we follow the two-state convention. V_i can be understood as the position of the receptor molecule.

¹The reference was mostly made to a publication which popularizes some rudiments of my idea [T. Duke and D. Bray, Proc. Nat. Acad. Sci. **96**, 10104 (1999)], and the attribution of credit was inappropriately made in T. Duke, N. Le Novère and D. Bray, J. Mol. Bio. **308**, 541 (2001).

Obviously, V_i is influenced by whether this receptor is bound to ligand. We suggest that V_i is also influenced by the conformational states of its nearest-neighboring receptors, due to receptor-receptor interaction which is dependent on their conformational states. We use H_i to represent whether there is a ligand binding, that is, $H_i = H \neq 0$ if the receptor is bound to ligand, otherwise $H_i = 0$. H can be understood as the force or torque generated by the ligand binding. HV_i is the energy due to ligand binding. Given the ambient chemical concentration $[L]$, the occupancy, i.e. the fraction of receptors with ligand bound, is

$$c = \frac{[L]}{[L] + K_d}, \quad (1)$$

where K_d is the dissociation constant. Therefore each H_i is randomly distributed between H and 0, with probabilities c and $1 - c$, respectively. Formally, the probability distribution can be written as

$$p(H_i) = c\delta(H_i - H) + (1 - c)\delta(H_i), \quad (2)$$

where $\delta(x) = 0$ if $x \neq 0$, while $\int \delta(x)dx = 1$. Since usually $K_d < [L]$, it is reasonable to regard the randomness of H_i as quenched [3,4].

We associate each site i with a variable n_i , which is 1 if there is a receptor on this site, and is 0 otherwise. In reality, of course V_i and H_i are only defined when there is a receptor on site i . However, because the presence or not of a receptor at site i has been determined by n_i , for the convenience of treatment, equivalently we may associate V_i and H_i to each site i , no matter whether there is a receptor on site i . Since the receptors are allowed to diffuse, n_i is time-dependent. One may constrain that the total number of receptors, $N = \sum_i n_i$, is conserved. It is also possible that the receptors may get out of the relevant region, hence N is not conserved. In any case, we may use grand canonical ensemble approach characterized by the chemical potential, which, in the first case, is given by the constraint on the conservation of receptor number.

Therefore, in absence of thermal fluctuation, we have

$$V_i = \psi\left(\sum_j t_{ij}n_jV_j + H_i - U_i\right), \text{ with } \psi(x) = \begin{cases} V^1 & \text{if } x > 0 \\ V^0 & \text{if } x \leq 0 \end{cases}, \quad (3)$$

where the summation is over the nearest neighbors j of site i , U_i is a threshold value, T_{ij} is a coefficient for the coupling between nearest neighbors. $\sum_j t_{ij}n_jV_j$ is the influence, i.e. force or torque, on the receptor at site i from the nearest-neighboring sites. H_i is the influence, i.e. force or torque, generated by the ligand binding of the receptor at i itself. Eq. (3) means that if the total influence on a receptor, as the sum of that due to its ligand binding and that due to the interaction with the nearest-neighbors, is larger than a certain value U_i , the receptors is in state V^1 . Otherwise it is V^0 . Equivalently, with $t_{ij} = t_{ji}$ and $t_{ii} = 0$, the dynamics is determined in the following Hamiltonian,

$$\mathcal{H} = -t \sum_{\langle ij \rangle} n_i n_j V_i V_j - \sum_i H_i n_i V_i + U \sum_i n_i V_i, \quad (4)$$

where $\langle ij \rangle$ represents pairs of nearest neighbors, t_{ij} and U_i have been set to be constants. Eq. (4) gives the total energy of the system, with the kinetic energy neglected. The first

term is the total interaction energy of nearest-neighboring pairs. The second term is the energy due to ligand binding. The third term is the original energy, which, together with the first term, determines the probability distribution of the conformational states of all the receptors in the absence of ligand binding of any receptor. Without ligand binding and coupling with others, the energy of a receptor is UV^0 for state V^0 , and is UV^1 for state V^1 . With ligand binding but without coupling, it is $(U - H)V^0$ for state V^0 , and is $(U - H)V^1$ for state V^1 . The interaction energy of the pair $\langle ij \rangle$, if sites i and j are both occupied by receptors, is $-tV_iV_j$, the value of which is dependent on both V_i and V_j . Since ligand binding can cause more V_i to flip from V^0 to V^1 , it is required that $(H - U)(V^1 - V^0) > 0$. Precise form of \mathcal{H} is subject to future experiments. However, (4) captures the essential elements while allows a theoretical treatment.

For convenience, we transform to a spin language, with the spin defined as

$$S_i = 2 \frac{V_i - V^0}{V^1 - V^0} - 1. \quad (5)$$

Hence it is $S_i = 1$ if $V_i = V^1$, and is $S_i = -1$ if $V_i = V^0$. In order to simplify the mathematics without loss of the essence, suppose $V^1 = -V^0$. Therefore we obtain the following simplified Hamiltonian

$$\mathcal{H} = -J \sum_{\langle ij \rangle} n_i n_j S_i S_j - \sum_i B_i n_i S_i, \quad (6)$$

with $J = t(V^1 - V^0)^2/4$, $B_i = (H_i - U)(V^1 - V^0)/2$, which is thus $B_1 = (H - U)(V^1 - V^0)/2$ with probability c and is $B_2 = -U(V^1 - V^0)/2$ with probability $1 - c$, i.e.

$$p(B_i) = c\delta(B_i - B_1) + (1 - c)\delta(B_i - B_2). \quad (7)$$

We call this model, defined by (6) and (7), floating Ising model with a quenched random field at each site. Note that it is not a diluted Ising model, since n_i is not a quenched disorder, but is a dynamical variable on the same footing as S_i .

It should be mentioned that a cell signalling process often involves different and largely separated time scales. On a much longer time scale the degrees of signalling and clustering evolve after the stimulus, till they return to the pre-stimulus levels. So there is a piece of short-time scale physics, and there is a piece of long-time scale physics. Here it suffices to discuss the short-time scale physics, i.e. the quasi-equilibrium part of the theory. With an incorporation of the feedback, the long-time scale behavior is similar to that described in Ref. [3,4]. With the adaptation process taken into account, the ligand concentration above should be replaced as the change of the ligand concentration. However, as studied in [3,4], perfect adaptation is equivalent to no ligand binding.

Guo and Levine studied the clustering of receptors under the assumption that the interaction between neighbors depends on whether the receptors are liganded [20], while the signalling problem was not considered. As described in our model, We think that it is more likely that the interaction depends on the conformations of the receptors, rather than whether the receptors are liganded. In addition to the consistency with the situation of chemotaxis [2-4], this is also supported by the strong experimental evidence that oligomerization is stabilized by receptor-receptor interaction promoted by conformational changes of the receptors [11-14,18,19].

3. MEAN-FIELD CALCULATION OF SIGNALLING

The activity of the system is $\langle \sum_i n_i V_i \rangle$, where the summation is over all the lattice sites, $\langle \dots \rangle$ represents ensemble average, i.e. average over the probability distribution of all possible configurations of the system. It is straightforward to write it as $(V^1 - V^0)W/2$, with $W = \langle \sum_i n_i S_i \rangle = Im$, where $m = \langle n_i S_i \rangle$ is the average spin per site, I is the number of lattice sites. The signalling can be characterized by the *change* of W when the occupancy is changed from $c = 0$ to c , i.e. $W(c) - W(c = 0) = I[m(c) - m(c = 0)]$.

Therefore we set out to calculate m as a function of c , using grand canonical ensemble approach. The grand partition function is

$$\mathcal{Z} = \prod_i \left(\sum_{n_i=0,1} \sum_{S_i=-1,1} \right) \left(\prod_i e^{\beta \mu \sum_i n_i} \right) e^{-\beta \mathcal{H}}, \quad (8)$$

where μ is the chemical potential, \mathcal{H} is as given in (6). Using Hubbard-Stratonovich transformation, we obtain

$$e^{-\beta \mathcal{H}} = A \int \left(\prod_i d\phi_i \right) \exp \left[-\frac{1}{2} \sum_{i,j} \phi_i K_{ij}^{-1} \phi_j + \sum_i (\phi_i + \beta B_i) n_i S_i \right]$$

where $A = (2\pi)^{-I/2} (\det K)^{-1/2}$. $K = \beta(J_{ij})$ is a matrix, K^{-1} is its inverse. Consequently

$$\mathcal{Z}(\{B_i\}) = A \int \left(\prod_i d\phi_i \right) \exp \left(-\frac{1}{2} \sum_{i,j} \phi_i K_{ij}^{-1} \phi_j \right) \prod_i [2 + e^{\beta \mu + \phi_i + \beta B_i} + e^{\beta \mu - \phi_i - \beta B_i}]. \quad (9)$$

Based on $\ln \mathcal{Z} = \lim_{n \rightarrow 0} (\mathcal{Z}^n - 1)/n$, the replica method yields

$$\ln \mathcal{Z}(\{B_i\}) = \lim_{n \rightarrow 0} \frac{1}{n} \left\{ A^n \int \left(\prod_{\alpha,i} d\phi_i^\alpha \right) \exp \left(-\frac{1}{2} \sum_{\alpha,i,j} \phi_i^\alpha K_{ij}^{-1} \phi_j^\alpha \right) \prod_{i,\alpha} [2 + e^{\beta \mu + \phi_i^\alpha + \beta B_i} + e^{\beta \mu - \phi_i^\alpha - \beta B_i}] - 1 \right\}, \quad (10)$$

where $\alpha = 1, \dots, n$ denote the replicas.

Averaging $\ln \mathcal{Z}$ over the B_i distribution (7), one obtains

$$\begin{aligned} \overline{\ln \mathcal{Z}} &= \int \ln \mathcal{Z}(\{B_i\}) \prod_i p(B_i) \prod_i dB_i \\ &= \lim_{n \rightarrow 0} \frac{1}{n} \left\{ A^n \int \left(\prod_{\alpha,i} d\phi_i^\alpha \right) \exp \left(-\frac{1}{2} \sum_{\alpha,i,j} \phi_i^\alpha K_{ij}^{-1} \phi_j^\alpha \right) \right. \\ &\quad \times \left. \prod_i \left[c_1 \prod_\alpha (2 + e^{\beta \mu + (\phi_i^\alpha + \beta B_1)} + e^{\beta \mu - (\phi_i^\alpha + \beta B_1)}) + c_2 \prod_\alpha (2 + e^{\beta \mu + (\phi_i^\alpha + \beta B_2)} + e^{\beta \mu - (\phi_i^\alpha + \beta B_2)}) \right] - 1 \right\}, \quad (11) \end{aligned}$$

where, for notation convenience, we have set $c_1 = c$, and $c_2 = 1 - c$.

The mean field value of ϕ_i^α can be obtained by deciding the saddle point of the integrand in Eq. (11), for which one can obtain

$$\sum_j K_{ij}^{-1} \Phi_n = \frac{c_1 f_1^{n-1} g_1 + c_2 f_2^{n-1} g_2}{c_1 f_1^n + c_2 f_2^n}, \quad (12)$$

where Φ_n is the saddle point value of ϕ_i^α , corresponding to n replicas, $f_k = 2 + e^{\beta\mu + (\Phi_n + \beta B_k)} + e^{\beta\mu - (\Phi_n + \beta B_k)}$, $g_k = e^{\beta\mu + (\Phi_n + \beta B_k)} - e^{\beta\mu - (\Phi_n + \beta B_k)}$, ($k = 1, 2$). Taking $n \rightarrow 0$ limit, we have $\sum_j K_{ij}^{-1} \Phi = c_1 g_1 / \bar{f}_1 + c_2 g_2 / \bar{f}_2$, where $\Phi = \lim_{n \rightarrow 0} \Phi_n$, $\bar{f}_k = 2 + e^{\beta\mu + (\Phi + \beta B_k)} + e^{\beta\mu - (\Phi + \beta B_k)}$, $\bar{g}_k = e^{\beta\mu + (\Phi + \beta B_k)} - e^{\beta\mu - (\Phi + \beta B_k)}$, ($k = 1, 2$). Therefore

$$\Phi = \beta\nu J \left(c_1 \frac{g_1}{\bar{f}_1} + c_2 \frac{g_2}{\bar{f}_2} \right). \quad (13)$$

is the mean-field value of the Hubbard-Stratonovich field. The saddle point approximation implies that

$$\overline{\ln \mathcal{Z}} = \lim_{n \rightarrow 0} \frac{A^n}{n} \left\{ \exp \left(-\frac{n}{2} \sum_{i,j} \Phi K_{ij}^{-1} \Phi \right) (c_1 \bar{f}_1^n + c_2 \bar{f}_2^n)^I - 1 \right\}. \quad (14)$$

m , as a measure of the activity, is calculated in the following way. For given $\{B_i\}$, the ensemble average of S_i is $\langle S_i \rangle_{\{B_i\}} = \partial \ln Z(\{B_i\}) / \partial (\beta B_i) = \partial \ln Z(\{B_i\}) / \partial (\phi_i) = \sum_j K_{ij}^{-1} \langle \phi_i \rangle_{\{B_i\}}$, whose average over $p(\{\{B_i\}\})$ gives the m . Thus $m = \overline{\langle S_i \rangle_{\{B_i\}}} = \sum_j K_{ij}^{-1} \Phi$, where Φ is the mean-field value of the Hubbard-Stratonovich field, as given in (13). Therefore $\Phi = \beta\nu J m$. Eq. (13) can thus be written as

$$m = \sum_{k=1,2} c_k \frac{e^{\beta\mu + \beta\nu J m + \beta B_k} - e^{\beta\mu - \beta\nu J m - \beta B_k}}{2 + e^{\beta\mu + \beta\nu J m + \beta B_k} + e^{\beta\mu - \beta\nu J m - \beta B_k}}. \quad (15)$$

The average receptor number is $\langle N \rangle = \partial \overline{\ln \mathcal{Z}} / \partial (\beta\mu)$, and can be evaluated as

$$\langle N \rangle = I \sum_{k=1,2} c_k \frac{e^{\beta\mu + \beta\nu J m + \beta B_k} + e^{\beta\mu - \beta\nu J m - \beta B_k}}{2 + e^{\beta\mu + \beta\nu J m + \beta B_k} + e^{\beta\mu - \beta\nu J m - \beta B_k}}. \quad (16)$$

(15) and (16) can also be obtained in a simple mean field approach regarding $H - \mu N$ as an effective Hamiltonian, and considering four possibilities of the state (n_i, S_i) at site i , subject to an effective field $\nu J m + B_i$.

Hence we have obtained an analytical expression for the signalling, as a statistical average quantity. If the receptor number is conserved in the system, (16) gives μ as a function of the receptor number.

Sensitivity of the signalling to ligand concentration is given by $\partial m / \partial c$, which can be arbitrarily large.

For annealed randomness, which may apply to the rare case in which $[L]$ is comparable to K_d , one may obtain

$$m = \frac{\sum_{k=1,2} c_k (e^{\beta\mu + \beta\nu J m + \beta B_k} - e^{\beta\mu - \beta\nu J m - \beta B_k})}{\sum_{k=1,2} c_k (2 + e^{\beta\mu + \beta\nu J m + \beta B_k} + e^{\beta\mu - \beta\nu J m - \beta B_k})}, \quad (17)$$

and

$$\langle N \rangle = I \frac{\sum_{k=1,2} c_k (e^{\beta\mu + \beta\nu J m + \beta B_k} + e^{\beta\mu - \beta\nu J m - \beta B_k})}{\sum_{k=1,2} c_k (2 + e^{\beta\mu + \beta\nu J m + \beta B_k} + e^{\beta\mu - \beta\nu J m - \beta B_k})}, \quad (18)$$

In the high temperature limit, the difference between (15) and (17), and that between (16) and (18) tend to diminish.

4. CLUSTERING

The conformation-dependent interaction not only enhances signalling sensitivity, it is also responsible for clustering. The first term of the Hamiltonian (6) implies that, in order to minimize the Hamiltonian, the receptors tend to aggregate together to maximize the number of nonzero $n_i n_j$ with $S_i = S_j$ for neighboring $\langle ij \rangle$. Therefore receptors with the same conformational state tend to cluster. However, because of thermal fluctuation, they cannot all cluster together, since there is a probability distribution for various configurations. As an illustration of the situation, a snapshot obtained in a Monte Carlo simulation is shown in Fig. 1.

The second term in (6) determines how ligand binding affects the clustering situation. It can be seen that U determines the bias of the distribution of the receptor state $\{S_i\}$ between 1 and -1 when there is no ligand binding ($H_i = 0$). That is, if $U = 0$, for not-too-strong interaction compared with the temperature $(\beta J)^2$, there is an equal number of receptors with spin 1 and those with -1 . If $U > 0$, there are more receptors with spin -1 . U also determines whether ligand binding enhances or suppresses the clustering. For example, we may consider two typical cases:

Case (i), $U = H$. According to (3), this means that merely a ligand binding of a receptor is enough to change its conformational state. In this case, $B_1 = 0$, $B_2 = -H(V^1 - V^0)/2$.

Case (ii), $U = 0$. According to (4), this means that in the absence of ligand binding ($H_i = 0$), there is no bias in the state distribution. In this case, $B_1 = H(V^1 - V^0)/2$, $B_2 = 0$.

These two cases can map to each other with the transformation $H \rightarrow -H$ and $c \rightarrow 1 - c$.

In case (i), since $B_2 < 0$, in the absence of ligand, all S_i tend to be close to -1 . Ligand binding causes more S_i to be 1. Because receptors with the same value of S_i tend to cluster, those with different S_i tend to be disconnected, the conclusion is that ligand binding suppresses clustering.

In case (ii), if there is no ligand binding, there is no biasing field, so there are equal probabilities for S_i to be 1 and -1 , consequently the clustering is minimized. When there is ligand binding, the clustering is enhanced.

For other values of U , i.e. $U \neq 0$ and $U \neq H$, we have the following general picture. Without ligand binding, there is a uniform field B_2 at every site. In the presence of ligand binding, there is a random field as given by (7). With a rough mean field estimation, one may see that whether the clustering is enhanced depends on whether $(1 - c)B_2 + cB_1$ has larger absolute value than $|B_2|$. Thus only if $H > 2U/c$, is the clustering enhanced by ligand binding. Case (ii) belongs to this region. If $U = H/2$, we have $B_1 = -B_2$.

The clustering of receptors can be studied quantitatively by defining a clustering correlation function as

$$C(r) = \frac{\langle n_i n_{i+r} \rangle - \langle n \rangle^2}{\langle n \rangle^2}, \quad (19)$$

where the average is over different sites i and different directions of \mathbf{r} first, and then over the thermodynamic ensemble, i.e. different possible configurations at the same temperature.

²There is no such a limitation if the adaptation on a long time scale is considered, see Ref. [3,4].

$\langle n \rangle = \langle N \rangle / I$ is the density of receptors on the lattice. If there is no clustering correlation, $\langle n_i n_{i+\mathbf{r}} \rangle = \langle n \rangle^2$, consequently $C(r) = 0$. This correlation function measures the deviation from the non-correlating case and allows comparison of situations for different receptor densities. This definition is similar to the two-point correlation function in the study of galaxy clustering. One may also define higher-order correlation functions. We leave for future investigations the analytical calculation of the clustering correlation function, and turn to numerical simulations here.

5. RESULTS OF MONTE CARLO SIMULATIONS

To investigate the extent of clustering and to calculate the activity, we have done Monte Carlo simulations using the Metropolis algorithm on a square lattice. In the simulations, we conserve the receptor numbers. We have specifically studied Case (ii), from which one may obtain the results for case (i) simply by changing c to $1 - c$. The results show strong correlations for small values of r and weak correlations for large values of r . The decay looks like exponential, as expected for a non-critical system.

We studied correlation functions for different values of the ligand binding fraction c , with a same receptor density $\langle n \rangle$. See Figs. 2(a) and 2(b) for results under different values of the coupling-noise-ratio βJ . From the correlation function for small values of r , it is clear that the larger the ligand binding fraction c , the larger the correlation. This confirms the above analyses.

Comparing Figs. 2(a) and 2(b), it can be seen that with larger coupling-noise ratio βJ , as in Fig 2(a), the correlation function $C(r)$ is larger at small values of r , while smaller at large values of r , indicating that clustering is stronger. On the other hand, for larger βJ , the decay of $C(r)$ with r is faster, because it is further from criticality.

We also studied the correlation functions for different values of receptor densities $\langle n \rangle$ with a same ligand binding fraction c (see Fig. 2(c)). It is shown that the smaller the receptor density, the larger the correlation function. This can be understood, since the larger the density, the less freedom two receptors can approach each other.

In the simulations, we also obtained the activity. The activity W can also be written as $\sum_i' S_i$, where \sum_i' represents summation over the receptors instead of the lattice sites. Hence it equals NM , where M is the average activity per receptor.

Fig. 3 gives the relation between the average activity per receptor M and the ligand binding occupancy c , for different values of coupling-noise ratio βJ . From the plots, we see that the activity increases with the ligand binding fraction c , with βJ , and with the receptor density $\langle n \rangle$, as consistent with the mean field solution.

6. SUMMARY AND DISCUSSION

In this paper, we propose a statistical mechanical theory which accounts for clustering and signalling of receptors in a same framework. Clustering and signalling of a network of receptors are treated as statistical average quantities. In our model, which can be reduced to a so-called floating Ising model in a random field, the interaction energy between neighboring receptors depends on their conformational states, therefore through this interaction,

the conformational state of one receptor influences those of its neighbors. On the other hand, since the receptors are allowed to move on the membrane, the receptors with a same conformational state tend to cluster together, in order to decrease the total energy of the system. Therefore clustering and signalling are unified as two consequences of the same coupling between receptors.

According to our theory, clustering exists even in absence of the ligand binding. This is consistent with a recent experimental finding [18]. We have studied clustering, based on an appropriate definition of clustering correlation function. Monte Carlo simulations were made. To obtain the situation that ligand binding enhances the clustering, a parameter, namely the threshold value U for the change of the conformational state, which determines the state distribution in the absence of ligand binding, must be within a certain range. If the values of the variable characterizing the two conformational states are symmetric, i.e. with a same magnitude and with opposite signs, then a simple possibility is that this threshold value is zero. We note that it was found experimentally that, in the absence of ligands, the receptors are hindered to cluster by certain inhibitors, which are squeezed out when ligands bind [21]. In such a case, it may be constrained that clustering is always enhanced by ligand binding.

By using a mean field theory based on Hubbard-Stratonovich transformation and replica method, as well as by using Monte Carlo simulations, we also studied the activity as a function of ligand binding fraction c .

This model is also be interesting from the point of the view of statistical mechanics. On the other hand, it is straightforward to make appropriate extensions of this model, for example, to put in more details of the realistic systems. The adaptation can be studied by straightforwardly generalizing our previous approach based on a counteracting field as a feedback from the signal to the field [3]. In this case, both signalling and clustering are adapted through a feedback, on a long time scale.

A simple and direct experimental test of our theory is to examine whether conformational state of a receptor can be changed by ligand binding of its nearest-neighboring receptors. It is interesting to study the forces generated by ligand bindings of a receptor and its neighbors. Parameters in our model need to be measured. The techniques used in studying single molecules may be useful for this subject.

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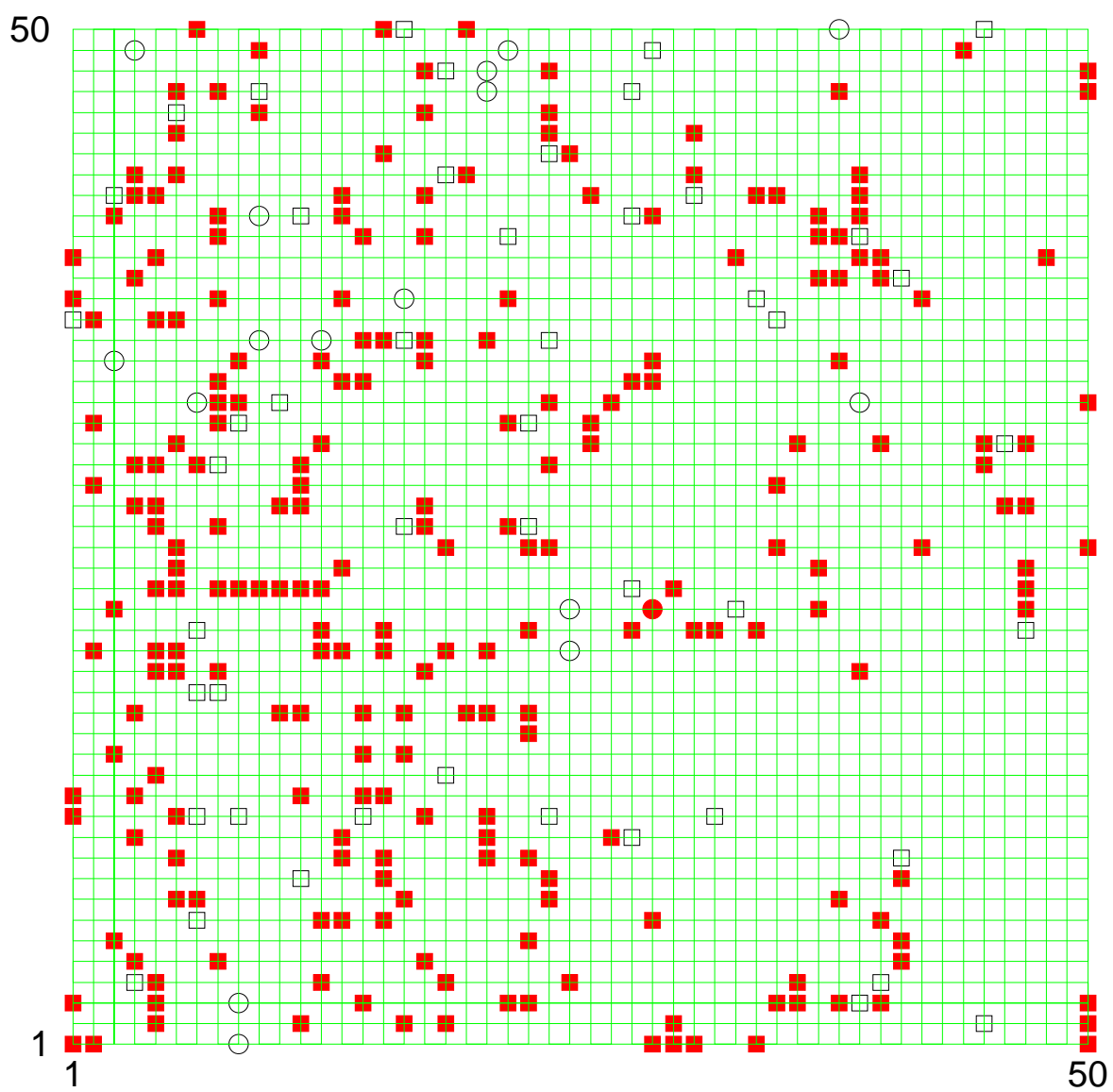
I am grateful to Prof. Howard Berg at Harvard University for enlightening, useful and pleasant discussions during my work on Ref. [4] and the current paper.

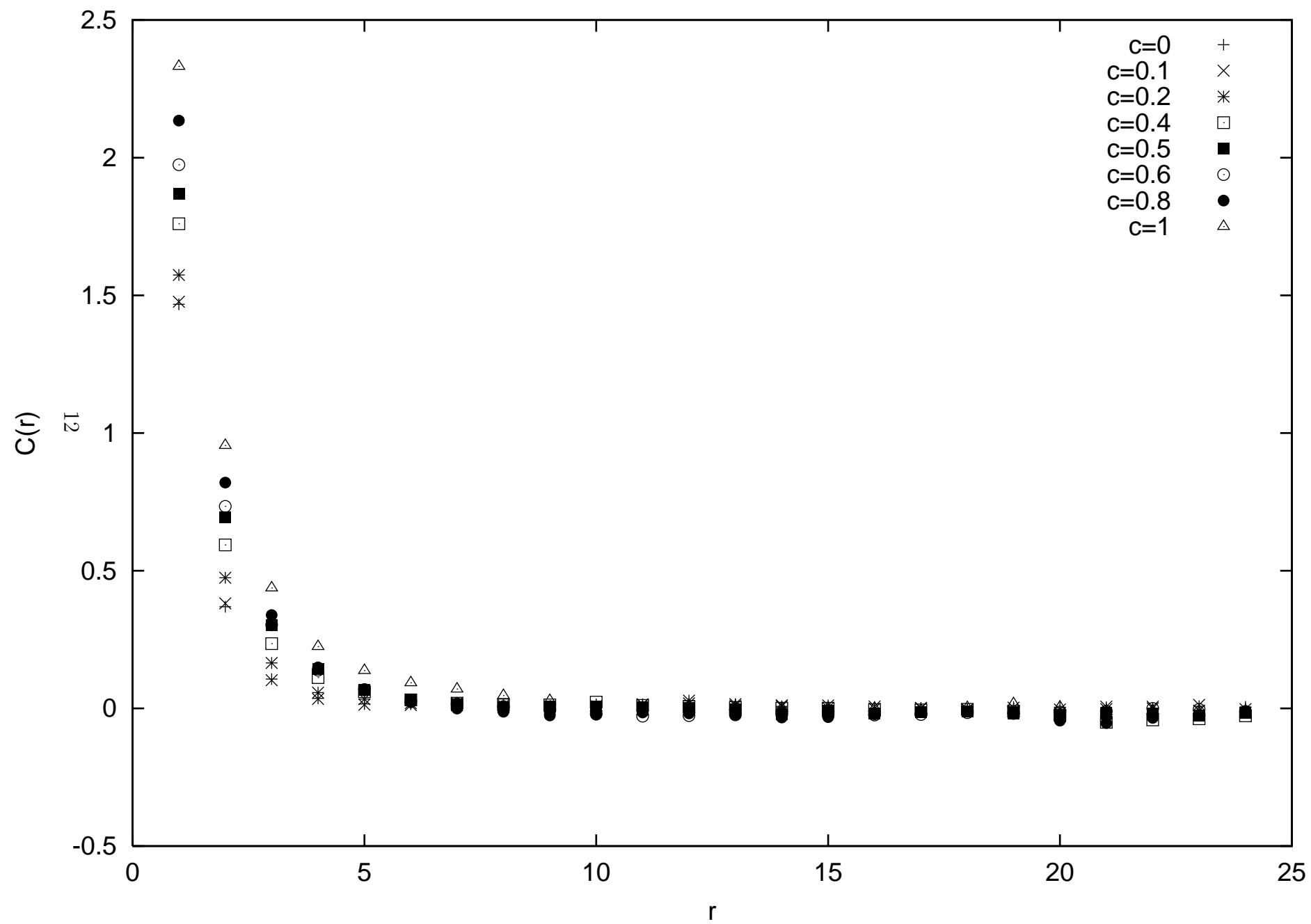
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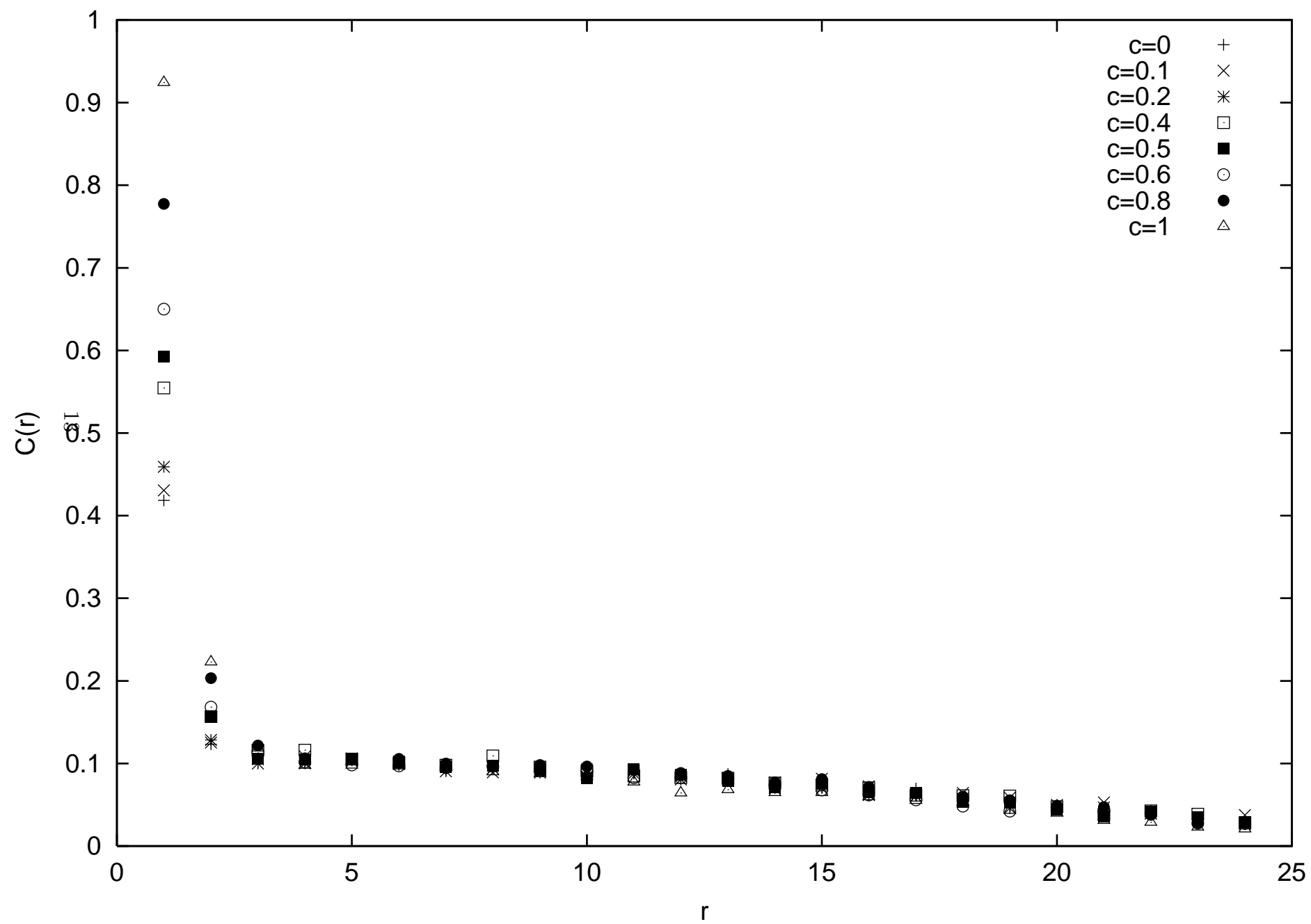
Fig 1. A configurational snapshot of 300 receptors on a 50×50 lattice. It is in 10000 Monte Carlo steps after an initial random configuration. An empty circle represents a receptor with $S_i = -1$ and $B_i = 0$, a filled circle represents a receptor with $S_i = -1$ and $B_i = B$, an empty square represents a receptor with $S_i = 1$ and $B_i = 0$, a filled square represents a receptor with $S_i = 1$ and $B_i = B$. The probability for B_i to be B is 0.8. $\beta B = 2$, $\beta J = 0.8$. Obviously, increasing βJ enhances clustering.

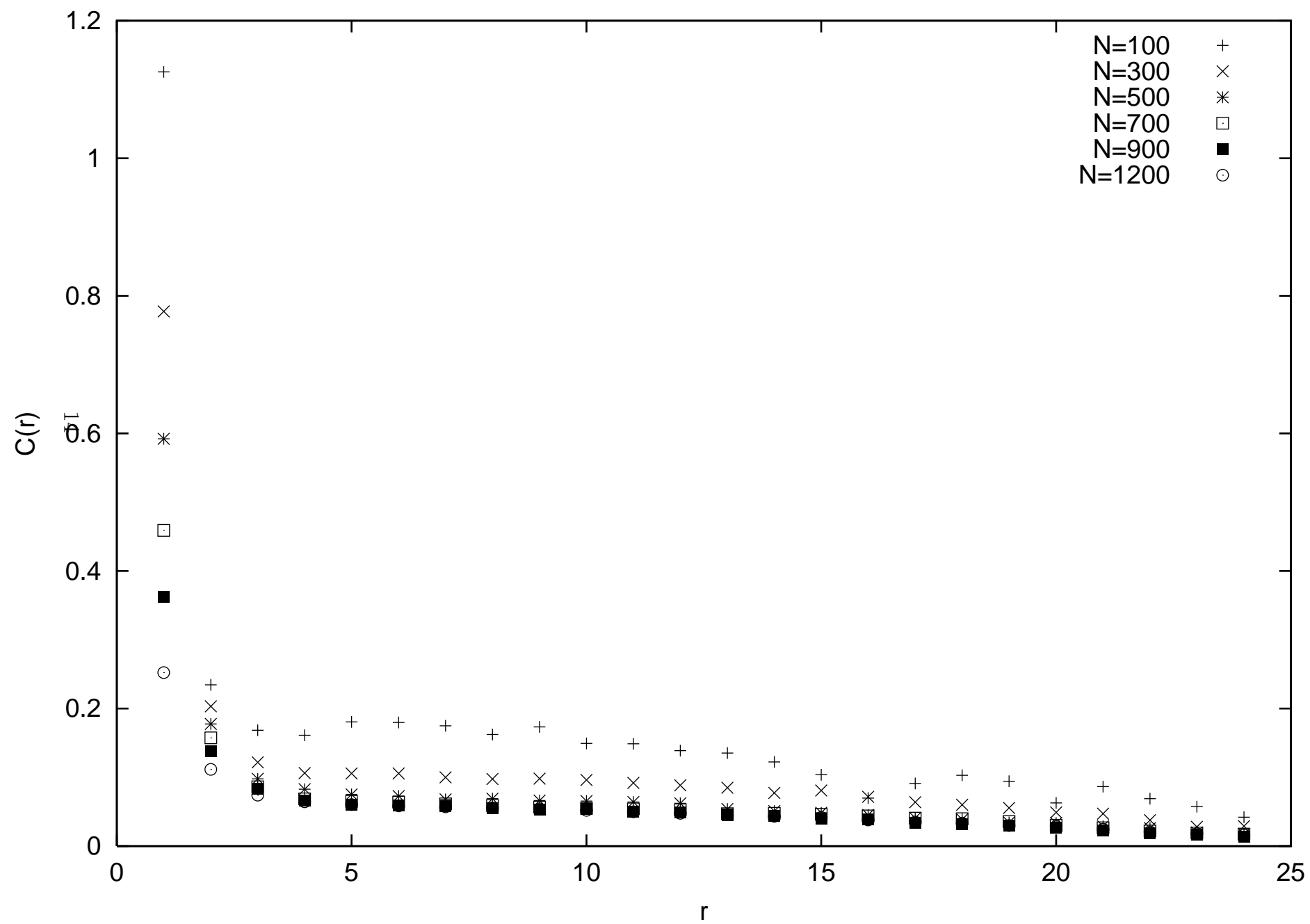
Fig 2. Monte Carlo calculation of the clustering correlation function $C(r)$ for receptors on a 50×50 lattice. The result is obtained by averaging over different sites with the same distance, and over 1000 Monte Carlo steps following 10000 initial steps to approach equilibrium. $\beta B = 2$. (a) $\beta J = 1.6$, there are 300 receptors, results for different values of ligand binding fraction are displayed as different symbols. (b) $\beta J = 0.8$, there are 300 receptors, results for different values of ligand binding fraction are displayed as different symbols. (c) $\beta J = 0.8$, the ligand binding fraction $c = 0.8$, results for different total numbers of receptors are displayed as different symbols.

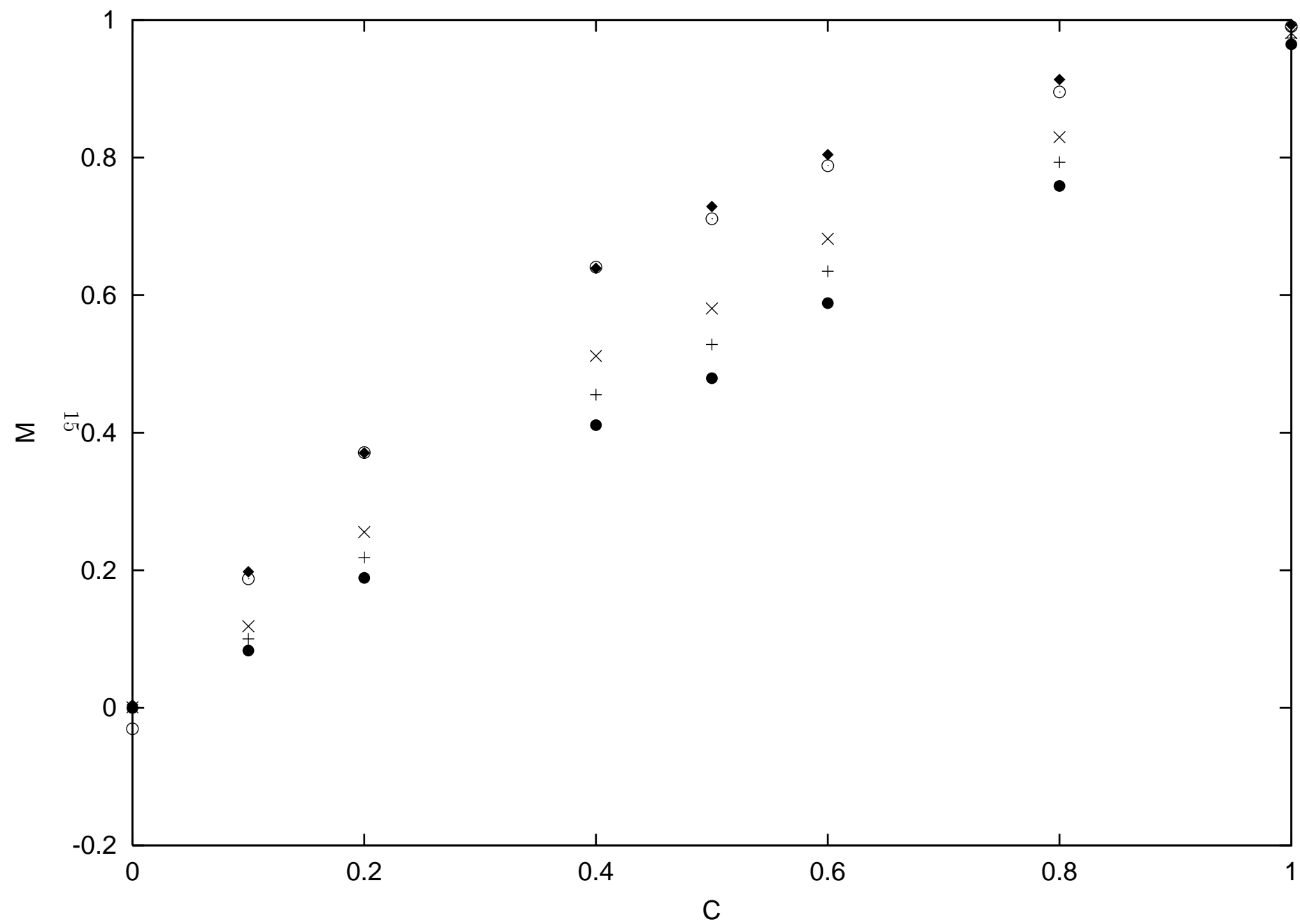
Fig3. Monte Carlo calculation of the activity of the receptors on a 50×50 lattice. For comparison between different densities of receptors, we give the relation between the average “spin” per receptor M and the ligand binding occupancy c . The result is obtained by averaging over all receptors and over 1000 Monte Carlo steps following 10000 initial steps to approach equilibrium. $\beta B = 2$. (i) $N = 300$, $\beta J = 0$ (\bullet), i.e. there is no receptor-receptor interaction. (ii) $N = 300$, $\beta J = 0.4$ ($+$). (iii) $N = 300$, $\beta J = 0.8$ (\times). (iv) $N = 300$, $\beta J = 1.6$ (\odot). In this case, the deviation of $M(c = 0)$ from 0 is spontaneous “magnetization” due to strong coupling, but it is always brought to 0 by the negative feedback on a long time scale [3,4]. (v) $N = 900$, $\beta J = 0.8$ (\diamond).











REFERENCES

- [1] H. Berg, *Random Walk in Biology* (Princeton University Press, Princeton, 1993); Phys. Today **53**(1), 24 (2000).
- [2] Y. Shi and T. Duke, Phys. Rev. E **58**, 6399 (1998); <http://xxx.arXiv.org/abs/physics/9901052>.
- [3] Y. Shi, Europhys. Lett., **50**, 113 (2000); <http://xxx.arXiv.org/abs/physics/9901053>.
- [4] Y. Shi, Phys. Rev. E **64**, 021910 (2001); <http://xxx.arXiv.org/abs/physics/0103033>.
- [5] S. Khan *et al.*, Biophys. J. **65**, 2368 (1993).
- [6] R. Jasuja *et. al*, Proc. Natl. Acad. Sci. USA, **96**, 11346 (1999).
- [7] R. Jasuja *et. al*, Biophys. J. **76**, 1706 (1999).
- [8] J. L. Spudich and D. E. Koshland, Proc. Natl. Acad. Sci. USA, **72**, 710 (1975).
- [9] J. A. Bornhorst and J. J. Falke, Biochemistry **39**, 9486 (2000).
- [10] V. Sourjik and H. C. Berg, Mol. Microbio., **37**, 740 (2000).
- [11] M. A. Lemmon *et. al*, Embo J., **16**, 281 (1997).
- [12] C. -H. Heldin, C.-H., Cell, **80**, 213 (1995).
- [13] M. A. Lemmon *et. al*, Trends Biochem. Sci., **19**, 459 (1994).
- [14] R. N. Germain, Curr. Biol. **7**, R640 (1997).
- [15] Z. Reich *et. al*, Nature, **387**, 617 (1997).
- [16] A. Ashkenazi and V. M. Dixit, Science **281**, 1305 (1998).
- [17] F. G. Giancotti and E. Ruoslahti, Science, **285**, 1028 (1999).
- [18] L. Gilboa *it et. al*, Mol. Bio. Cell, **11**, 1023 (2000).
- [19] A. Schulz, A. *et. al*, J. Bio. Chem., **275**, 2381 (2000).
- [20] C. Guo and H. Levine, Biophys. J., **77**, 2358 (1999).
- [21] Y. Jiang, *et. al*, Science **283**, 543 (1999)